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Functional nucleic acid lateral flow magnetic biosensor based on blocking the super PCR and magnetic test strip for rapid detection of genetically modified maize MON810†

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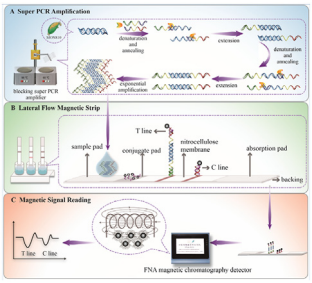
HIGHLIGHTS

- A high-speed, super-sensitive, highly stable functional nucleic acid magnetic test strip is developed
- Magnetic signal readout is anti-background interference, visual and high stability
- The use of super PCR device shortens the target amplification time to 5 min
- Blocking the super PCR and magnetic probe achieve the ssDNA/dsDNA conversion of PCR
- 5-min blocking super PCR and 5-min magnetic signal readout reduce the screening time to 10 min
- Exponential PCR and magnetic sensitization improve the sensitivity to a single copy

GRAPHICAL ABSTRACT



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ABSTRACT

Although PCR is a typical temperature-variable amplification technique for nucleic acids, it still faces challenges in rapid screening, such as low speed, poor sensitivity, inferior visualization, and weak stability. In this paper, a universal functional nucleic acid (FNA) lateral flow magnetic biosensor was constructed using blocking super PCR (BS-PCR) and a magnetic test strip (MTS). In theory, the visualized and magnetic output of dsDNA-based amplicons were achieved via ssDNA/dsDNA conversion by blocking linkers in the PCR primers and magnetic probes. In application, high-speed, super-sensitive, highly stable rapid screening was realized by taking advantage of the speediness of the super PCR reactor and the anti-background interference, visualization and high stability of magnetic signal readout. The genetically modified maize MON810 was selected as a double-stranded model target, while 5-min BS-PCR and 5-min magnetic signal readouts achieved the screening results within 10 min. Furthermore, the

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Lateral flow biosensor
Rapid screening

exponential PCR amplification and magnetic-based sensibilization improved the sensitivity to a single copy. This biosensor is simple and portable, with significant potential for rapid on-site screening.

1. Introduction

Developed in 1983, PCR is a typical nucleic acid amplification technique. The trace nucleic acid fragment can be cloned millions of times *in vitro*, while the purity requirements of the samples are low [1]. Due to its high specificity, good sensitivity and simple operation process, PCR has been used in various fields such as food safety inspection and supervision. However, PCR is not suitable for rapid on-site screening since it often takes over 1 h to complete 30 to 40 cyclic amplifications. In addition, the amplified products consisted of double strands are often analyzed using the gel electrophoresis. Although significant efforts have been made to reduce the amplification time [2–5], higher speed, easier operation and stabler long-term signal readings are in demand for rapid on-site screening.

The lateral flow biosensor displays high detection accuracy while it is convenient and efficient. It is a visual analytical method developed to realize rapid screening [6]. Compared with antibody-based immunochromatography test strips, the functional nucleic acids (FNA)-based lateral flow biosensor are applied more comprehensively, exhibiting higher stability, with low cost [7–9]. However, various factors limit the development of the FNA test strips. First, it is difficult to achieve the visualizable and highly-sensitive detection of dsDNA amplicons when in combination with the conventional amplification methods, such as PCR, LAMP, and RPA. Second, the elimination of background interference is limited by the intrinsic properties of signal molecules, biological samples, and the environment [10]. Most test strips used fluorescent probes, such as Cy3/Cy5 organic dyes, lanthanide chelates, and quantum dots, suffer from autofluorescence interference, resulting in a low signal to noise ratio [11]. The third involves the stability of signal molecules [12]. Therefore, developing a novel FNA test strip platform is necessary to visualize dsDNA amplicons, with little background interference, and high signal reading stability.

The advantages of nanoscale magnetic materials provide many exciting application opportunities. First, the magnetic signal readout is resistant to background interference [13]. Highly sensitive measurements can be carried in cloudy or visually blurred samples without further pretreatment [14]. Furthermore, magnetic nanoparticles (MNPs) can provide a qualitative visual output based on their aggregation-induced brown color while allowing accurate analysis via their superparamagnetic signal response. The signal intensity of the magnetic field exhibits a direct linear relationship with the number of MNPs. Third, the magnetic signal displays high stability and an extended decay period, establishing a stable and robust screening platform [15]. MNPs have been widely used in various fields, including biomedicine [16], environmental protection [17], and food safety [18]. However, reports on MNP probes-based FNA lateral flow biosensors (FNALFB) with magnetic signal as output are lacking.

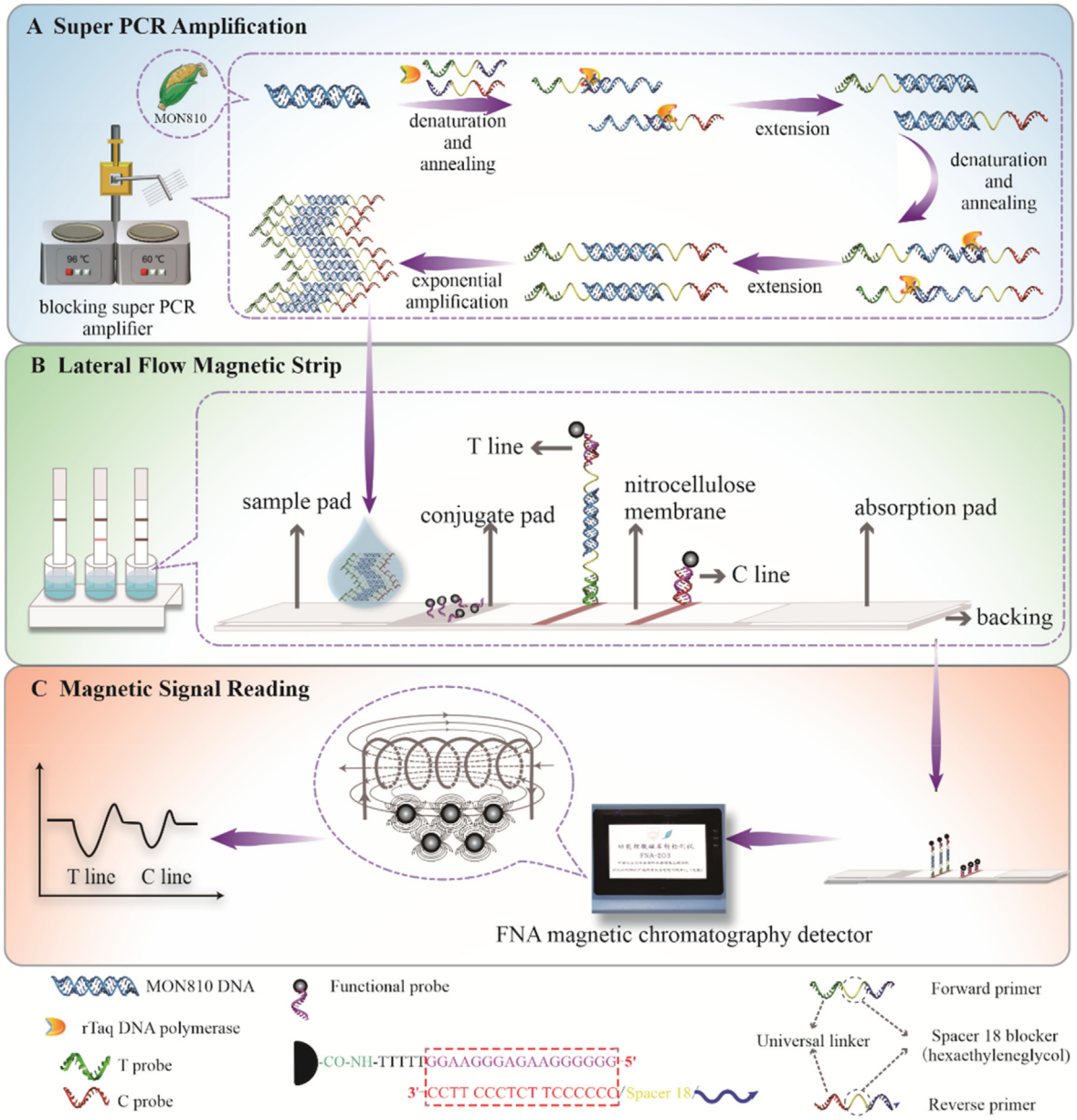
Since the commercialization of genetically modified (GM) crops began in 1996, the planting area and the research scope of GM crops have been continuously expanded and increased [19]. GM crops bring huge economic benefits and ecological benefits, but their potential risks are also controversial. Up to now, more than 50 countries have established strict legal and labelling systems for the safety management of genetically modified organisms (GMOs) [20]. The Chinese government attaches great importance to the management of GMOs and has formulated GMOs labeling rules [21]. Event-specific GMO detection faces ever-increasing challenges, due to the sheer number of GMOs entering the marketplace. Therefore, it is very important to establish a rapid and accurate genetic modification detection system.

To address these theoretical and practical issues, we constructed a universal FNA lateral flow magnetic test strip (MTS) for the first time. The genetically modified maize MON810 was used as a model target for the biosensor that was fabricated by combining the MTS and blocking super PCR (BS-PCR) (Scheme 1). In this strategy, the universal single-stranded oligonucleotide sequences (universal linker) are introduced into the 5' ends of the amplification primers using the Spacer 18 blockers, allowing the ssDNA/dsDNA conversion of the PCR product and the subsequent visualization and magnetic signal analysis. The super PCR reaction device can improve the amplification efficiency, completing 30 PCR cycles within 5 min. The single-stranded oligonucleotides matching the universal linker sequence are modified on the surface of MNPs to construct the magnetic signal probes used in the test strip. The BS-PCR products are also analyzed in 5 min using MTS. The magnetic signal probes provide qualitative visual output based on their aggregation-induced brown color while allowing accurate analysis of the magnetic field intensity via their superparamagnetic signal response. The magnetic signal output has the stability of long-term repeated signal reading. Exponential PCR amplification and magnetic-based sensibilization improve the detection sensitivity of MON810 to a single copy. Furthermore, this method does not rely on complicated equipment, while it is quick and easy to operate, meeting the requirements for the rapid on-site screening of targets.

2. Materials and methods

2.1. Materials

All nucleic acid sequences used in this study were synthesized by Sangon Biotech Co., Ltd (Shanghai, China). The details of the sequences are listed in Table S1. Furthermore, the plant Genomic DNA Kit was purchased from Tiangen Biotech (Beijing) Co., Ltd. The 200 nm carboxy modified MNPs (5 mg/mL) were purchased from ZhongKeLeiMing DaoJin Technology Co., Ltd (Beijing, China). The materials for preparing the test strip were purchased from Millipore (Bedford, MA, USA). The 10 × PCR buffer (20 mM Mg^{2+} Plus), dNTP mixture (10 mM each), and rTaq DNA polymerase were purchased from TaKaRa (Beijing, China). Trihydroxymethyl aminomethane (Tris), sucrose, Tween-20, Triton X-100, Tris-HCl, bovine serum albumin (BSA), sodium chloride–sodium citrate (SSC) buffer (20 concentrate, pH 7.0), $Na_3PO_4 \cdot 12H_2O$, 2-(N-Morpholino)ethanesulfonic acid hydrate (MES), N-Hydroxysuccinimide (NHS), and 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) were purchased from Sigma Chemical Company (St. Louis, MO, USA). The laboratory set up the super PCR device. The FNA magnetic chromatography detector was provided by Jiehao Scientific Instrument Co., Ltd (Shanghai, China).



Scheme 1. The universal FNALFB Based on BS-PCR and Magnetic Test Strips for the Rapid Screening of MON810.

2.2. Methods

2.2.1. Preparation of the MNP-ssDNA functional probes

The ssDNA-modified MNPs functional probes used to construct the MTS in this study were prepared using the amino-carboxyl chemical coupling method via the following procedures: EDC and NHS solutions were prepared at a concentration of 50 mg/mL with 25 mM pH 6 MES buffer, and stored at 4 °C in the dark for later use. The carboxy-modified MNPs were washed several times with 25 mM MES buffer and resuspended. Equal amounts of EDC and NHS were added to the resuspension and incubated at 37 °C for 1 h. After separation with a magnetic stand, the upper liquid was discarded, and the MNPs were washed several times with MES buffer and resuspended again. A specific amount of amino-modified ssDNA complementary to the universal linker in the reverse blocking primer was added to the activated MNP suspension and incubated on a rotator at room temperature for 1 h. The MNPs were washed with MES buffer to remove the remaining uncoupled nucleic acids and then resuspended with a complex solution (20 nM $Na_3PO_4 \cdot 12H_2O$, 5% BSA, 0.25% Tween-20, and 10% sucrose). The functional probes were prepared and stored at 4 °C in the dark.

2.2.2. Zeta potential and dynamic light scattering (DLS)

The zeta potential and dynamic light scattering (DLS) were measured using a Zetasizer Nano ZS analyzer. The instrument was preheated for 30 min before use. Unmodified MNPs, carboxyl activated MNPs, and ssDNA-modified MNPs at a 10 mg/mL concentration were diluted until optically clear. Then, 1 mL of the clear solution was added into the zeta potential or particle size sample cell (cleaned with ultrapure water before use), no bubbles present in the container. The sample cell was placed into the analyzer, after which the measurement was performed with default parameters.

2.2.3. Construction of the magnetic test strip

Here, 1 mg/mL streptavidin and 100 μM of the capture probe 1 or capture probe 2 solutions were mixed in equal amounts, after which the mixture was incubated at room temperature for 3 h to obtain the streptavidin-biotinylated capture probe complex. The FNALFB consists of five components: the sample pad, the conjugate pad, the nitrocellulose membrane, the absorbent pad, and the backing. The nitrocellulose membrane was fixed to the backing, after which pretreated capture probes 1 and 2, modified with streptavidin, were sprayed onto the nitrocellulose membrane to form a test line (T line) and a control line (C line) using a BioDot BioJet BJQ 3000 dispenser (Irvine, CA). The nitrocellulose membrane was left to dry naturally at room temperature. The different pads were assembled in a specific order on the backing with an overlap of approximately 2 mm to ensure that the solution could migrate through the test strip. Finally, the LFBs were cut at a width of 3.5 mm for testing using a Bio-Dot Paper Cutter module CM4000 (Irvine, CA).

2.2.4. Fabrication of the BS-PCR

The super PCR prototype device was set up, primarily consisting of five parts [22,23]. (1) The real-time temperature monitoring system: A thermocouple (T-type, HXW) centered in a dedicated control tube was used to measure the temperature. The thermocouple amplification and linearization were transmitted using a temperature transmitter (SBWR-2260, K, Yuancheng) and was performed using an Arduino UNO v1.0 chip. The analog signal was digitized using an Arduino UNO chip and processed with Arduino IDE (version 1.8.1). (2) Ultrathin PCR reaction tube (Light Cycler capillaries, 20 μL, 04 929 292 001, Roche): This container had the function of fast heat conduction to realize a rapid change in sample temperature. (3) A stepper motor (42JSF630AS-1000, Just Motion Control): This device was powered by switching the power supply (S-100-24, Elecall) and controlled using an AC Servo Motor Driver (YZ-ACSD608, Moving) and LabVIEW Software (version 2014), which could control the movement of the robotic arm. (4) Two temperature-difference vessels (HH-1, Junsi): These vessels were used to control the temperature of the reaction system at 96 °C for denaturation and 60 °C for annealing and extension. (5) A control computer: This was used to monitor the temperature change in the reaction system and control the stepping motor.

The 20 μL BS-PCR systems were spun down to the bottom of each reaction tube via brief centrifugation and fixed to the robotic arm. The BS-PCRs were performed at 96 °C for 4 s and 60 °C for 6 s in one thermal cycle, and completed 30 cycles in 5 min. After amplification, the BS-PCR products were analyzed by FNA-MTSs.

2.2.5. Detection of GM maize MON810

Here, 2.5 μL of the BS-PCR product was mixed with 100 μL of running buffer (4 × SSC, 2% BSA, 0.05% Tween-20, 10 mM Tris-HCl and 0.002% TrtionX-100, pH 7.4). Then, 2 μL of the 10 mg/mL functional probe solution was loaded onto the conjugate pad. The sample pad of the test strip was inserted into the running buffer to initiate the reaction, during which the products migrated toward the absorption pad under capillary force, hybridizing with the functional probes on the conjugate pad to form composites. Subsequently, the composites migrated through the T line and C line, after which the MNPs accumulated to form brown bands at these lines. The test strip was removed, and the presence of the product in the solution was visually detected in 5 min. The magnetic signal was read out using an FNA magnetic chromatography detector after drying.

2.2.6. Sensitivity determination

The genomic mass concentration was measured using a microultraviolet analyzer. The following equation was used to convert ng/μL into copy numbers. MON810 samples at genomic concentrations of 2×10^4 copies, 2×10^3 copies, 2×10^2 copies, 2×10^1 copies, 2 copies, and 1 copy were prepared and tested according to the optimal parameters.

$$Copy\ number = \frac{concentration\ (ng/\mu L) \times volume\ (\mu L)}{haploid\ genome\ quality\ (ng)}$$

3. Results and discussion

3.1. Design principle of the biosensor

The universal biosensor is constructed in this study to detect a double-stranded MON810 target model which is integrated with two techniques, BS-PCR and FNA lateral flow MTS (Scheme 1). BS-PCR is used for target recognition and amplification, while MTS is used for signal conversion and output. The traditional PCR primers are designed to connect single-stranded oligonucleotides at the 5' ends via Spacer 18 blockers. The blockers can prevent the action of the polymerase for the forward amplification of the strand. The oligonucleotide segment (universal linker) is universal with signal conversion capabilities, and can be kept unchanged for different detection targets by simply replacing the specific primer section. Due to the effect of the blocker in the primer during BS-PCR, the strand extension terminates at the blocker, preventing the universal linker from being amplified and thus causing it to remain in a single-stranded state. Therefore, many double-stranded products with single-stranded nucleic acids at both ends are obtained within 5 min. Due to the universal linkers at both ends, the ssDNA modified on the MNP surfaces, and the capture probes assembled on the test strip are matched with each other. When the BS-PCR products flow upward with running buffer on the test strip via capillary force, the functional probes on the conjugate pad are hybridized with the universal linkers at one end of the products, allowing products to carry MNPs. The products continue to flow upward through the T line, allowing the capture probe 1 on the T line to hybridize with the universal linkers at the other end of the products to capture products with MNPs. The remaining functional probes that are not bound to the products continue to flow upward through the C line and are captured by probe 2. MNPs aggregation eventually allows the brown color on the T and C lines to become visible to the naked eye. The T line color is examined to determine whether the sample contains the target substance. When the MTS is dry, the specific magnetic signal strength on the T line is accurately measured using an FNA magnetic chromatography detector.

3.2. Verification of the carboxyl-amino coupling method

The binding between streptavidin and biotin has long been regarded as the strongest non-covalent biological interaction [24]. This bond forms rapidly and remains stable in a wide pH and temperature range [25]. Biotin is an organic substance, while streptavidin is a protein. This research used a covalent coupling method where a carboxyl and an amino group form the amide bond. The oligonucleotide modified with an amino group at the 3' end was coupled with magnetic Fe_3O_4 nanoparticles modified with carboxyl groups to form the functional probe required for MTS. The carboxyl-amino method is less expensive than the streptavidin-biotin coupling method while keeping highly stable.

Two techniques were used to characterize MNPs. One is zeta potential, a standard method for characterizing the surface potential of particles. The other is DLS, which is applied to characterize

the size and particle size distribution of nanoparticles. The zeta potential results indicated that carboxyl-modified MNPs exhibited a negative surface charge in the aqueous solutions (Fig. 1). After nucleic acid modification, the zeta potential decreased significantly due to the negatively charged phosphate backbone of the nucleic acid chain. The average particle size of the carboxyl-modified MNPs measured by DLS was 217.1 nm, but increased to 245.5 nm after coupling (Table 1). Therefore, the single-stranded nucleic acids were successfully coupled to the surfaces of the MNPs.

3.3. Solutions to the false positives produced by the magnetic test strip

The generation of false positives is a common problem in detection, and this situation also occurred in the lateral flow MTS constructed in this study. For buffer containing no BS-PCR product, a light brown color visible to the naked eye could also appear on the T line of the MTS. The results show that the false-positive signal on the T line declined in conjunction with continuous decrease of functional probe concentration. However, reducing the functional probe concentration to eliminate false positives resulted in reduced visibility and sensitivity of MTS. Whereas, due to the blocking effect of BSA on MNPs surface, the false-positive band on the T line could be effectively eliminated as the storage time at 4 °C increased. The false positive completely disappeared after 7 d (Fig. S1-Fig. S4). Therefore, the newly prepared functional probes were stored at 4 °C in the dark for one week before use.

3.4. Design and screening of the primer-specific binding sequence

Before BS-PCR, the reasonable design of the target-specific binding sequence in the primer is pivotal and is related to the feasibility, sensitivity, and specificity of the experiment. Primer design follows many principles, including primer length, melting temperature, G/C content, secondary structures between the primer itself and the upstream and downstream primers, and the size of amplification fragments. Furthermore, to obtain primer pairs with the best amplification effect in this study, the primer design was achieved using professional software (DNAMAN) according to the specific sequence of the insect-resistant maize MON810 transformant based on the national standard.

Four pairs of selected primers were subjected to BS-PCR, and the products were analyzed using 2% agarose gel electrophoresis. The four pairs of primers were F1 and R1, F2 and R2, F3 and R3, and F3 and R1 (see Table S1 for primer sequences). The Tm were 59.4 °C and 59.2 °C, 58.7 °C and 58.6 °C, 59.4 °C and 59.4 °C, and 59.4 °C and 59.2 °C. The amplified fragment lengths were 33 bp, 77 bp, 66 bp, and 81 bp, respectively. Fig. S5 (a) shows that the F1 and R1 primers displayed the brightest amplified products, followed by F3 and R1. Since the F1 and R1 primer pair partially overlapped in sequences, the 9-nt nucleotides at the 3' ends of the upstream and downstream primers were complementary (Fig. S5 (b)), amplification was performed without the template, showing a false-positive and bright product band. Therefore, the F3 and R1 primers were ultimately selected to design the blocking primers with the universal linkers for subsequent experiments.

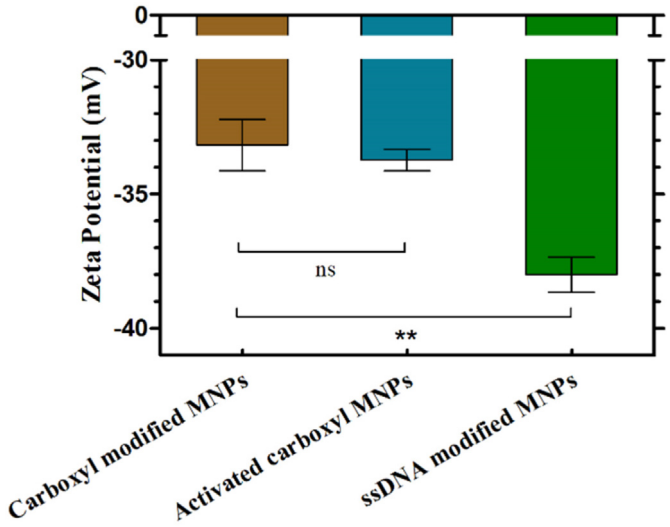


Fig. 1. Results of Zeta potential.

Table 1
The particle size of the functional probe.

Types	Z-Average (nm)	PDI
Carboxyl modified MNPs	217.1	0.193
Activated carboxyl MNPs	224.0	0.197
ssDNA modified MNPs	245.5	0.232

Notes: Z-average represents the average particle size of magnetic nanoparticles. PDI represents the dispersion coefficient. When PDI value is between 0.08 and 0.7, the dispersion of the system is considered as the best application range of the algorithm.

3.5. Feasibility of the biosensor

BS-PCR was used in this biosensor for target recognition and amplification, while FNA-MTS realized signal conversion and output. Fig. 2 (a) illustrates the 2% agarose gel electrophoresis of the BS-PCR amplification products using standard and blocking primers, indicating that the amplification of sample without target was not obvious. With the addition of blocking primers, the molecular weight of the amplification product was higher than the ordinary primers due to the universal linkers in the primers. Moreover, the product with both forward and reverse primers with universal linkers was larger than those with only one of the primers with a linker. The BS-PCR products shown in Fig. 2 (a) were analyzed via MTSs illustrated in Fig. 2 (b), showing that a dark brown color appeared on the T line only when the target was present in the sample and both the forward and the reverse primers were connected with universal linkers. Furthermore, as the concentration of BS-PCR product in line 5 of Fig. 2 (a) decreased, the brown color at the T line gradually faded from dark to light and finally disappeared (Fig. S6 (a)). A linear relationship was evident between the magnetic signal intensity on the T line and the product concentration (Fig. S6 (b)). These results indicated that the universal linkers were successfully introduced into the double-stranded BS-PCR product and analyzed using FNA-MTS, the universal FNA lateral flow MTS based on BS-PCR for detecting MON810 was feasible.

3.6. Optimization of the experimental condition

3.6.1. Primer concentration in the BS-PCR system

The super PCR contained the same components in its amplification system as the traditional PCR. However, to reduce the time required for the test, the primer concentration and DNA polymerase concentration in the super PCR system were remarkably improved. In this study, the primer also had a blocker and a universal linker in addition to the target-specific binding sequence. When the primer concentration was too high, the primer dimers were easily generated, which was not conducive to amplification, with reduced efficiency, and increased cost. When the

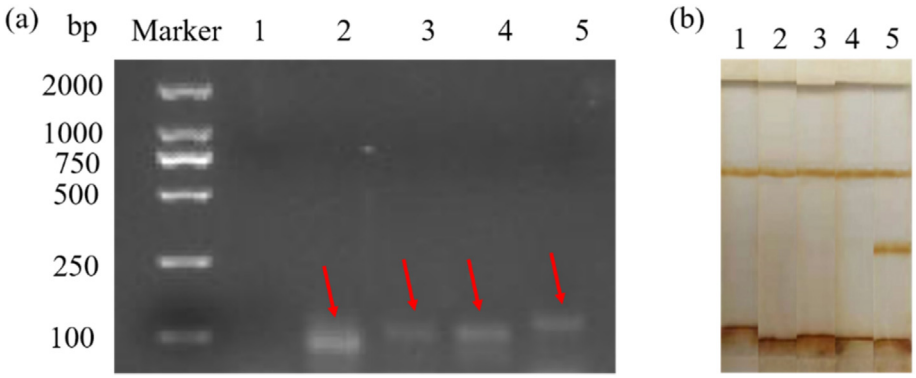


Fig. 2. Verification results of the primer design.
(a) BS-PCR amplification results (1: negative control without target and with blocking forward and reverse primers, 2: ordinary primers, 3: blocking forward primer and ordinary reverse primer, 4: ordinary forward primer and blocking reverse primer, 5: blocking forward and reverse primers). (b) The detection results of the FNA lateral flow MTS (1–5 correspond to 1 to 5 in (a)).

concentration was too low, the collision efficiency between the molecules declined. Therefore, it was necessary to select the proper primer concentration to ensure the highest amplification efficiency. A higher primer concentration increased the number of BS-PCR products, while the color on the T line darkened, and the magnetic signal strength enhanced. At the concentration of 2 μM, these growth trends tended to be flat (Fig. S7). Therefore, 2 μM was carefully selected for further experiment.

3.6.2. Concentration ratio of MNPs to ssDNA

During the MNP-ssDNA functional probe preparation, the concentration ratio was considered crucial as it influenced the density of the single-stranded nucleic acids on the MNP surfaces. A low nucleic acid density on the MNP surfaces might cause the ssDNA to be in a tortile or lodging state, rather than a vertical state, which was not conducive to complementary pairing with the amplified products in short time and reduced the detection sensitivity of the lateral flow test strip. A nucleic acid density that was too high also obstructed its binding to the products due to the influence of steric hindrance, reducing sensitivity and increasing cost. Therefore, in this study, the concentration ratio of MNPs to ssDNA was optimized. The concentration of carboxylated magnetic Fe_3O_4 remained unchanged, while the aminated ssDNA concentration was reduced continuously, allowing the ongoing increase of the concentration ratio: 2: 1, 2.5: 1, 3: 1, and 3.5: 1 (mg/mL: μM). When the concentration ratio was 2.5: 1, the T line displayed the darkest brown color and the strongest magnetic signal (Fig. S8). Therefore, 2.5: 1 (mg/mL: μM) was chosen as the optimal ratio for the preparation of the functional probes.

3.6.3. The functional probe concentration on the conjugate pad

The functional probe concentration on the conjugate pad was also an important factor affecting the sensitivity. Excessively high concentration of MNPs were prone to aggregation, hindering their diffusion on the test strip. However, low concentrations prevented the BS-PCR products from binding to the functional probes. Therefore, the MNPs captured on the T line did not display a high enough concentration, while the color was not clear, resulting in poor detection sensitivity. When the functional probe concentration was optimized, the volume on the conjugate pad remained unchanged, decreasing the concentration (10 mg/mL, 5 mg/mL, 2.5 mg/mL, 1.25 mg/mL, and 0.625 mg/mL) for analysis. The results are shown in Fig. S9. The increase in the concentration caused the color at the T line to become darker, with enhanced magnetic signal intensity. The color was deepest and the magnetic signal was strongest when the concentration was at 10 mg/mL. Furthermore, a trailing phenomenon appeared on the test strip due to the high MNP concentration, rendering the test strip not clean enough. However, for a concentration of 10 mg/mL, the color on the T and C lines is sufficient to be visible to the naked eye. Therefore, 10 mg/mL was selected for the follow-up experiments.

3.7. Sensitivity analysis

The biosensor was examined and optimized during its construction. Its sensitivity was tested to target the MON810 genome. Fig. 3(a) indicates the qualitative visual output results of FNA-MTS for the BS-PCR products. Fig. 3(b-c) displays the precise magnetic signal intensities, indicating the differences in magnetic signal strength between the experimental group and the control on the test strips as a function of the genome concentration logarithm. When the genome concentration was a single copy, the visible brown color also appeared at the T line, and the magnetic signal intensity was high, which was significantly different from the control. Therefore, the magnetic signal detection sensitivity of this strategy was as low as a single copy (the sensitivity comparison is listed in Table S5).

3.8. Specificity verification

Detection specificity is a vital reference point for a detection method. GM maize MIR604 and GA21, GM soy 87705 and 87769, and GM rape RF3 were selected as non-specific targets to explore the specificity of the strategy constructed in this study. During the analysis, the non-specific GM crop genomes concentrations were 100 times that of the GM maize MON810, in which the genome concentration of MON810 was about 2.5×10^2 copies, while that of other genetically modified crops was about 2.5×10^4 copies. Fig. 4 indicates that only the MON810 exhibited a dark brown band that could be clearly distinguished by the naked eye at the T line, and the magnetic signal intensity was much higher than that of other groups. Although GA21 and MON87769 did produce signals at the T lines, their signals were much weaker than that of MON810, and were close to the signal of the blank control sample in the sensitivity analysis and weaker than the signal of the single copy MON810 sample. Therefore, the slight signals produced by GA21 and MON87769 were less likely to be confused with the signals produced by specific target at low concentrations, and were not easy to cause false positive results. This strategy displayed high specificity, which has practical application value.

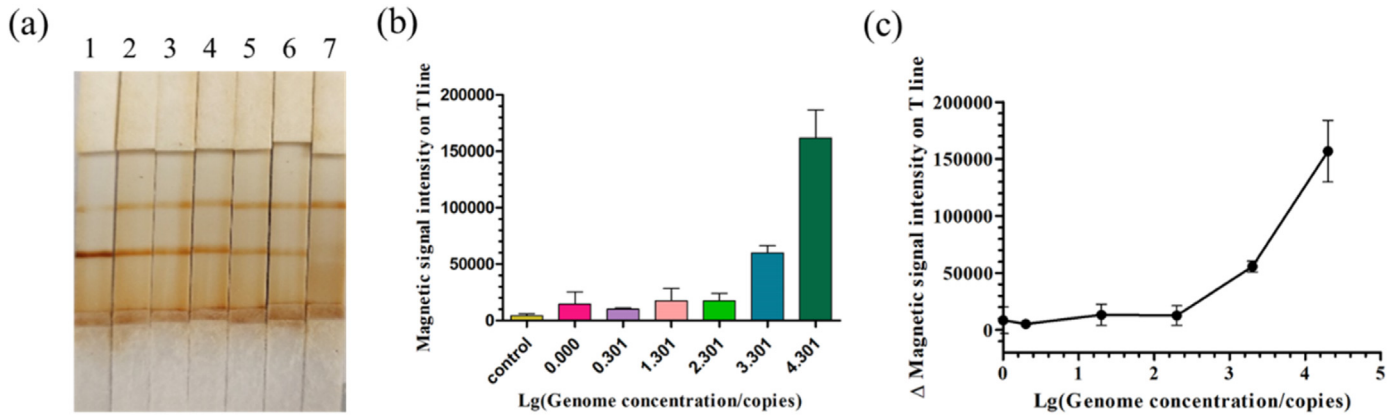


Fig. 3. The results of the sensitivity analysis.
(a) The depth of the color on the T line changes with the genome concentration (the genome concentrations from 1 to 6: 2×10^4 copies, 2×10^3 copies, 2×10^2 copies, 2×10^1 copies, 2 copies, and 1 copy, 7 is control). (b) The histogram of the T line magnetic signal strength corresponding to the logarithm of each genome concentration. (c) The difference in magnetic signal strength between the experimental and control groups on the T line varies in conjunction with the logarithm of the genome concentration. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

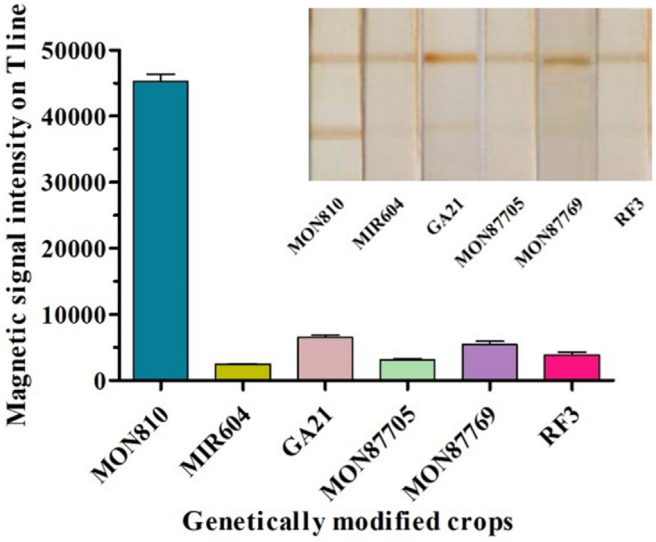


Fig. 4. The results of specificity analysis.

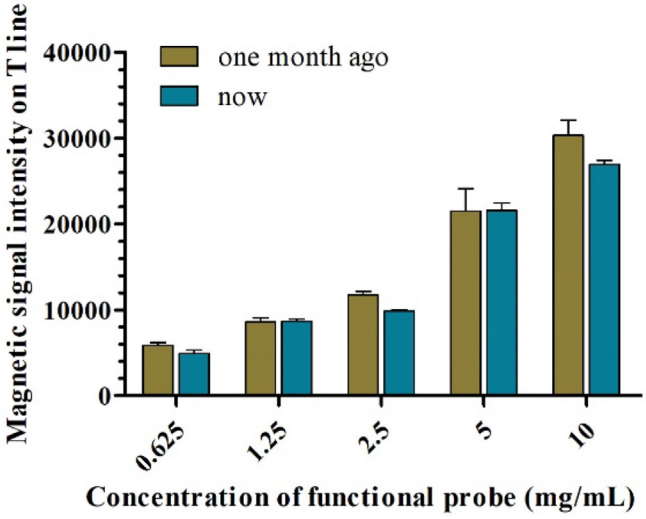


Fig. 5. Verification results of the magnetic signal stability.

3.9. Verification of the magnetic signal stability

The stability of the magnetic signal was further verified to reflect the superiority of the MTSs. The MTSs used to optimize the concentration of the functional probes on the conjugate pad were stored at room temperature for one month. Then, the magnetic signal intensities on the T line were re-measured using an FNA magnetic chromatography detector. The measured results were compared with those obtained a month earlier, revealing that the intensities of the measured magnetic signal did not change significantly (Fig. 5). And the magnetic signal intensities were measured six consecutive times at concentrations of 10 mg/mL, 5 mg/mL, and 2.5 mg/mL. The relative standard deviations were calculated, which were all lower than 5% (Table 2). Therefore, using an FNA magnetic chromatography detector to measure the magnetic signal is highly accurate. Using the magnetic signal as a signal output displays excellent stability.

Table 2
Accuracy verification of the magnetic chromatography detector.

Concentration of functional probe (mg/mL)	Magnetic signal on T line (n = 6)	RSD (%)
2.5	9449.41	4.89%
5	21002.53	4.16%
10	26489.45	2.39%

4. Conclusion

In conclusion, a universal FNA lateral flow MTS is constructed for the first time using MNPs as the signal output element, which was combined with super PCR to develop a biosensor for rapid screening of double-stranded targets. In this study, a covalent coupling method is used with bonded carboxyl and amino groups to prepare signal probes, which is inexpensive and easy to store. With MON810 as the double-stranded model target, 5-min BS-PCR

is used for target identification and amplification, and 5-min magnetic chromatography is used for signal conversion and output. The entire detection time is only 10 min. Exponential PCR amplification and magnetic-based sensibilization improve the sensitivity to a single copy. This strategy exhibits significant application potential for rapid on-site screening. Furthermore, the newly constructed visual FNA-MTS is fast and super-sensitive, displaying excellent stability for long-term repeated magnetic signal reads. This study further promotes the rapid detection technology of the FNALFB with the magnetic signal as the signal output. It provides a foundation for further in-depth research and the development of related technologies.

CRediT authorship contribution statement

Shuting Li: Methodology, Investigation, Writing – original draft, Writing – review & editing, and, Software. **Jingjing Tian:** Methodology, and, Validation. **Longjiao Zhu:** Investigation, and, Writing – review & editing. **Kunlun Huang:** Resources, and, Supervision. **Huashuo Chu:** Software, and, Visualization. **Wentao Xu:** Resources, Supervision, Conceptualization, Writing – review & editing, and, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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